Synthesis of Piperazinylalkylisoxazoline Analogues and Their Binding Affinities for Dopamine Receptor Subtypes

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In recent years, extensive efforts have been made to explore potent ligands for dopamine D₁ or D₂ receptor for the discovery of antipsychotic drugs.¹ In this connection, some of us have recently reported the design and synthesis of a piperazinylalkylisoxazole library (Figure 1), of which some ligands were found to exhibit high binding affinity and selectivity for the D₁ receptor over the D₂ receptor.² In continuation of this research program, we have also been interested in the construction of a structurally similar piperazinylalkylisoxazoline library. We envisaged that the slightly different structural feature of isoxazoline moiety may affect the physicochemical properties of molecules in the library and thus alter their binding affinities with dopaminergic receptors. With this envision in mind and careful scrutiny on binding affinities of piperazinylalkylisoxazole analogues, we designed a focused library of piperazinylalkylisoxazoline derivatives where n = 3 or 4 (Figure 1). Herein, we wish to report the synthesis of piperazinylalkylisoxazoline compounds and their binding affinities for dopamine receptor subtypes.

Our synthetic strategy to the construction of a library of piperazinylalkylisoxazolines was quite similar to that adopted for the synthesis of piperazinylalkylisoxazoles.³ Based on the solution phase combinatorial reductive amination of isoxazoline aldehydes with piperazine derivatives, synthesis of piperazinylalkylisoxazolines was quite straightforward.

The preparation of starting isoxazoline aldehydes was described in Scheme 1. Treatment of aldehydes 1 with hydroxylamine hydrochloride and sodium carbonate in aqueous ethanol (EtOH : H₂O = 2/1) provided the corresponding oximes 2 in 90%-100% yields. Oximes 2 were reacted with N-chlorosuccinimide (NCS) in the presence of catalytic amount of pyridine in THF at 60 °C under nitrogen atmosphere. The reaction mixtures were cooled to room temperature over 30 min and then 4-penten-1-ol (for n = 3) or 5-hexen-1-ol (for n = 4) was added slowly. The mixture was treated with triethylamine (Et₃N). In situ generation of nitrile oxides and their 1,3-dipolar cycloadditions proceeded to give the cyclized alcohols 3 in 42%-66% yields. PCC oxidation of alcohols 3 in the presence of silica gel in CH₂Cl₂ afforded isoxazoline aldehydes 4 in 45%-75% yields (Scheme 1).

Combinatorial synthesis of piperazinylalkylisoxazolines was accomplished by the reductive amination of the prepared isoxazoline aldehydes 4 with a variety of commercial phenylpiperazine derivatives 5 using NaBH(OAc)₃,⁸ as outlined in Scheme 2. To solutions of aldehydes 4 and compound 5, NaBH(OAc)₃ was added in THF at room temperature. The reaction mixtures were stirred for 1 h, and the solvent was evaporated. The crude product was purified by silica gel column chromatography.

Scheme 1. Reagents and Reaction Conditions: i) NH₂OH·HCl, Na₂CO₃, 60 °C, 1 h, EtOH/H₂O (2/1), 90-100%. ii) pyridine (cat.), NCS, 60 °C, 0.5 h, THF/4-penten-1-ol (for n = 3) or 5-hexen-1-ol (for n = 4), Et₃N, 50 °C ~ r.t., 2 h, 42-66%. iii) PCC, SiO₂, CH₂Cl₂, 5-12 h, 45-75%.
amines 5 in CH₂Cl₂ were added NaBH(OAc)₃ (3 eq.) and molecular sieve (3 beads). And the reaction mixtures were stirred for 5-12 h at room temperature. After carrying out the aqueous workup, the reaction mixture in 2 mL of diethyl ether was treated with ethereal HCl. The precipitants were washed with diethyl ether and dried. Yields were 85%-95%.

The HCl salts of the products 6 were precipitated. The precipitants were filtered, washed with diethyl ether, and dried in vacuo. All the products were obtained in good yields (85-95%) and high purities ranging from 85 to 93%. For the solution phase combinatorial screening, while in piperaizinylalkylisoxazoline series compounds for the dopamine receptors were also highly dependent on the length of the alkyl chain linker connecting two heterocycles and the substitution pattern at α-position on the phenyl group such as fluoro- (Ar₁5), chloro- (Ar₁6), methyl- (Ar₁2), methoxy- (Ar₁3), and ethoxy- (Ar₁4) were mainly employed, considering the binding results of piperaizinylalkylisoxazoline series. Dihethylmethylpiperaazines such as 1-(diphenylmethyl)piperazone (Ar₁8), 1-(4-chlorobenzhydryl)piperazone (Ar₁9) and 1-bis(4-fluorophenyl)-methylpiperazone (Ar₁10) were also employed (Figure 2).

The purities and identities of products were confirmed by ¹H NMR, HPLC and HRMS analysis after the conversion of HCl salts of products into the corresponding free amine products. Thus, a small focused library of the well-characterized 100 members was constructed by using reductive amination reaction (Scheme 2, Figure 2).

The constructed piperaizinylalkylisoxazoline library members were evaluated in vitro for dopamine D₂, D₃, D₄ receptors binding affinity by measuring their ability to displace radioligands ([³H]piperone for D₂ and D₃, [³H]YM-09151-2 for D₄) from the cloned human dopamine receptors D₂, D₃, and D₄ which were stably expressed in CHO cells, respectively. The affinity and selectivity of these compounds for the dopamine receptors were also highly dependent on the length of the alkyl chain linker connecting the phenyl group and the substitution pattern at α-position on phenylpiperaizinyl group as those of piperaizinylalkylisoxazoline analogues were. In this series, affinities of compounds with the alkyl chain length of n = 4 were low and showed low selectivities among receptors in the primary screening, while in piperaizinylalkylisoxazoline series compounds with a three atom ether (n = 3) showed low binding affinities and low selectivities among receptors. In other words binding affinities for two different libraries with the isoxazolé and isoxazolé structures showed the reverse pattern for the length of alkyl chain from n = 3 to n = 4. In addition, the synthesized library was isolated as racemic compounds with the stereogenic center. Table 1 shows the binding data of the selected compounds that exhibited good binding affinity and selectivity among dopamine D₂, D₃, and D₄ receptors. Compounds 6i, 6c, and 6d, with the methyl group at α-position of the phenyl group of Ar₁4, showed relatively low binding affinity for the D₂ (110-140 nM) and D₃ receptors (310-590 nM). With the introduction of methoxy (Ar₁3) and ethoxy (Ar₁4) group at α-position of phenyl group, the binding affinity to both dopamine D₂ and D₃ receptors increased (compounds 6e-6h). Compound 6e showed high affinity for the D₂ receptor (5 nM) with a 288-fold selectivity over the D₃ receptor. Compound 6g displayed high affinity value of 5 nM at the D₄ receptor with a 22-fold selectivity over the D₂ receptor. Especially, compound 6h exhibited good binding affinity (4 nM) at both D₂ and D₃ receptors with a 118-fold selectivity over the D₂ receptor. Introductions of electron withdrawing substituents such as fluoro and chloro groups (Ar₁5 and Ar₁6) at α-position of the phenylpiperazone did not give satisfactory binding values. Thus, compounds 6i-6m showed the moderate binding affinity to the D₃ receptor (21 nM-95 nM). Compound 6n,
with the methoxy group at \( m \)-position of the phenyl group (Ar,7), showed a slightly lower binding affinity (18 nM) than compound 6c with the methoxy group at \( \alpha \)-position of the phenyl group (Ar,3) (5 nM). It seemed that an introduction of alkoxy group at \( \alpha \)-position of the phenyl group is desirable for high binding affinity and selectivity at the D\(_2\) and D\(_{3}\) receptors over D\(_{1}\) receptor. Diphenylmethyppiperazine analogues 6d-6q displayed moderate to low binding affinities. Among them, compound 6q, with symmetric 1-[bis(4-fluorophenyl)methyl]piperazinyl group (Ar,10), displayed the slightly higher affinity than compounds 6c (Ar,8) and 6p with asymmetric 1-(4-chlorobenzhydryl)-piperazinyl group (Ar,9), to both D\(_2\) and D\(_{3}\) receptors. In general, most of compounds exhibited the high selectivity of both the D\(_2\) (3.5 to 465-fold) and D\(_{3}\) receptors (7.9 to 130-fold) over D\(_1\) receptor. However, the selectivity of the D\(_3\) receptor over D\(_2\) receptor was not significant (maximum of 6-fold selectivity). As for substituteds at the isoxazoline ring (Ar,2), dimethoxy (Ar,2) and 3-thienyl (Ar,3) groups seem to guarantee high affinity (Table 1 and Figure 2).

In summary, a small focused library of piperazinylalkylisoxazolines was constructed through solution phase combinatorial synthesis and observed for binding affinity at dopamine D\(_2\), D\(_{3}\), and D\(_1\) receptors. With the linker chain length of \( n \)-3 connecting two heterocycles, most of compounds exhibited good binding affinity and selectivity at the desirable target receptors, the D\(_2\) and D\(_{3}\) receptors over D\(_1\) receptor. It seemed that an introduction of alkoxy group at \( \alpha \)-position of the phenyl group (Ar,) guaranteed high binding affinities for the D\(_2\) and D\(_{3}\) receptors and high selectivity at the D\(_2\) and D\(_{3}\) receptors over D\(_1\) receptor. Compounds 6e and 6h showed IC\(_{50}\) values of 5 nM and 4 nM for the D\(_{3}\) receptor with 288-fold and 118-fold selectivity over D\(_2\) receptor, respectively. For the D\(_{2}\) receptor, they displayed binding affinities of 23 nM and 4 nM with 63-fold and 118-fold selectivity over D\(_2\) receptor, respectively.

### Experimental Section

Typical procedure for the construction of library members (6): To a solution of 3-[3-(3,4-dimethoxyphenyl)-4,5-dihydroisoxazol-5-yl]propanal (25.3 mg, 0.096 mmol) and 1-[2-(methoxyphenyl)piperazine (20.0 mg, 0.087 mmol) in dry CHCl\(_3\) (2 mL) were added NaH(OAc)\(_2\) (55.5 mg, 0.262 mmol) and molecular sieve (3 beads). And the reaction mixtures were stirred for 12 h at room temperature. Saturated NaHCO\(_3\) solution was added and the mixture was extracted with diethyl ether. Organic extracts were dried over anhydrous MgSO\(_4\) and concentrated. The residue was dissolved in 2 mL of diethyl ether and followed by treatment with 1M HCl solution in diethyl ether. The HCl salt of the product 6e was precipitated. The precipitant was filtered, washed with diethyl ether, and dried in vacuo. In this way the HCl salt of the product 6e was obtained as white solid (32.7 mg, 86%). Other compounds were synthesized analogously and the spectroscopic data of selected compounds were as follows.

#### Compound 6e: \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 1.76 (m, 6H), 2.53 (t, 2H), 2.73 (d, 3H), 2.99 (dd, 1H, J = 8.4 Hz, J = 10.8 Hz), 3.13 (s, 3H), 3.41 (dd, 1H, J = 10.4 Hz, J = 16.2 Hz), 3.87 (s, 3H), 3.93 (s, 6H), 4.78 (m, 1H), 6.96 (m, 6H), 7.41 (s, 1H); IR (CHCl\(_3\)) 2924, 2822, 1602, 1512, 1448, 1358, 1240, 1144, 1074, 918, 820, 761, 696 cm\(^{-1}\).

#### Compound 6g: \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 1.26 (q, 2H), 1.44 (t, 3H), 1.73 (br, 4H), 2.27 (t, 2H), 2.61 (d, 3H), 2.98 (dd, 1H, J = 7.8 Hz, J = 10.8 Hz), 3.14 (s, 6H), 3.28 (m, 1H), 3.92 (s, 6H), 4.11 (t, 2H), 4.77 (m, 1H), 6.94 (m, 6H), 7.40 (s, 1H); IR (CHCl\(_3\)) 2938, 2816, 1600, 1518, 1458, 1424, 1370, 1340, 1242, 1153, 1142, 1028, 1008, 916, 808, 752, 664, 628 cm\(^{-1}\).

#### Compound 6h: \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 1.23 (t, 1H), 1.47 (t, 3H), 1.78 (br, 5H), 2.49 (t, 1H), 2.70 (br, 3H), 3.02 (m, 1H), 3.15 (s, 4H), 3.44 (m, 1H), 4.08 (q, 2H), 4.75 (m, 1H), 6.97 (m, 4H), 7.07 (t, 1H), 7.20 (d, 1H), 7.39 (d, 1H); IR (CHCl\(_3\)) 2940, 2814, 1592, 1500, 1446, 1378, 1304, 1240, 1124, 1044, 908, 834, 750, 710 cm\(^{-1}\).

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### References


9. The related topic for the synthesis and stereochemistry of chiral ligands from isoxazoline library will be published as a title of ‘Asymmetric Synthesis of Chiral Pipenzinylpropylixazoline Ligands for Dopamine Receptors’.